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# THE RELATION OF ALGÆ TO DISSOLVED OXYGEN AND CARBON - DIOXIDE. WITH SPECIAL REFERENCE TO CARBONATES.

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## INTRODUCTION AND REVIEW OF LITERATURE.

During the summer months of 1908 and 1909, while associated with the Puget Sound Marine Station at Friday Harbor, Wn., I undertook the study of some algæ growing in the tide pools and lower tide levels of Puget Sound. The study was suggested by Dr. T. C. Frye, Professor of Botany at the University of Washington, and sought to find an explanation for the great variations already noted by him, in *Prionitis lyallii* as found at different levels or exposed to different degrees of light, heat, and possibly to different amounts of dissolved gases.

My observations included a considerable range of conditions as shown along the shores of the different islands, with all possible degrees of exposure to light, air and tidal currents. There is a range of over ten feet from lowest to highest tide levels, and a range of 10° F. in temperature, viz., from 53° to 63° F.; the pools left at high tide or those filled by spray, being about ten degrees warmer than the main body of water, which is very constant at 53° F.

Here in these upper levels, in this warmer water, is the favorite haunt of the typical form of *Prionitis,* where it is smaller and more branched than at the lower levels. This was at first supposed to be an adaptation to a smaller supply of $CO_2$ in the warmer pools. The warmer water has a lower coefficient of absorption for the gases, oxygen and $CO_2$. The more branched and divided forms would present more absorptive surface to compensate for the smaller amount of gas. Being more exposed to the sun than those much deeper in the water, the tougher, darker surface seemed to be a protective adaptation to these conditions, while the shorter, more compact and much-divided fronds could better resist the strain of the rough waves to which they are exposed in these higher positions. Whether the dark brown of the topmost plants, as opposed to the yellowish tinge of the lowermost ones, could be an adaptation to the different light intensities was not considered. The theory and explanation of Gaidukov ('04) and Blackman ('04) would lend some plausibility to such a view. Although *Prionitis* is left by the receding tide it is not required to resist drying out, as is the case in *Fucus* and its associates, for *Prionitis* is always covered by water, even in the highest pools. It is likely that there is some concentration of dissolved salts, due to evaporation, in such pools during low tide; but this, however, must be very slight in so short a time.

As it appeared on further investigation, however, none of the above explanations seemed adequate. The more luxuriant, though less typical growth, was found in places little exposed to strong light or increased temperature, and the best growth, with fronds least dissected, was found in a small bay where the water was the warmest and the exposure to sunlight the greatest. But this, like the other stations showing the most vigorous growth, had the advantage of constant currents to renew the supply of $CO_2$ and other foods, while affording a safe, protected position for attachment.

The fact, also, that young plants were always found starting around the rim of the pools where $CO_2$ would supposedly be abundant, but never on the bottom, where both oxygen and $CO_2$ would, in all probability, be absent or much diminished, though affording safer points of attachment, led to the belief that the whole problem would resolve itself into a question of aëration. The best growth was found at places best aërated, regardless of their position as to light, heat, or point of attachment, except as this might afford protection from rough waves or currents. Where currents were most constant, if not too violent, bringing a supply of $CO_2$ and removing the excreted oxygen, an important factor in the growth of aquatic plants, there the largest growth was found, both as to size of individuals and total bulk of plant growth.

Lacking facilities at the time for measuring the gas content of the water, that point was necessarily left unsettled, and the problem, so far as that region is concerned, was dropped indefinitely. Some two years later, this phase of the problem was taken up at the Missouri Botanical Garden. Although the conditions are quite different in small bodies of fresh water from those just cited in larger bodies of salt water, yet Birge and Juday ('11) find results in Wisconsin lakes similar to those obtained by Lebedinzeff in the Caspian and Black seas, and the same problem, in general, presents itself for solution, viz.: what is the relation of dissolved gases to algæ or submerged aquatics; first, as to life and death, or continuity; second, as to periodicity or rate of reproduction; third, as to habit, or morphologic adaptation to environment?

Pfeffer ('00) remarks that currents of water are of the utmost importance in providing aquatic plants with continual supplies of dissolved carbonic acid. Owing to the marked solubility of the latter a given volume of water can hold a higher percentage than is present in the supernatant air, while still more may be held in the form of dissolved carbonates. Indeed, according to Hassak ('88), a green plant is not only able to obtain carbon-dioxide from calcium bicarbonate, $Ca(HCO_3)_2$, but also can make use of 70% of the carbon-dioxide contained in sodium bicarbonate. This agrees with the investigations of Darwin and Pertz ('96), who showed by laboratory experiments that in water that is kept stirred, photosynthesis is about twice as vigorous as in water that is allowed to remain quiet. This had to do only with the removal of excreted oxygen, whereby its accumulation in the plant tissues is prevented. This fact added to the benefits derived from a constant renewal of $CO_2$ would be an important factor in the plant economy.

Dr. A. Disato ('08), after an investigation along the shore of the Mediterranean during the summer of 1907, says: "It is significant that the $CO_2$ content of the air over the sea and that over the land are the same (3 parts in 10,000), although the conditions are so different. Further, the $CO_2$ content of the sea air in the same region remains constant for a number of years by day as well as by night. He accepts the theory of Schloessing to account for the phenomenon. According to this, the $CO_2$ of the air and the carbonates and bicarbonates of the sea remain in equilibrium. The sea serves as a regulator of the $CO_2$ content of the air. By lessening the $CO_2$ pressure of the air, new carbonic acid comes from bicarbonates of the sea. On the other hand, by increased pressure, $CO_2$ is absorbed and more carbonates and bicarbonates are formed in the water. The constancy is further effected through transportation by the wind.

Dr. Disato made further investigations as to the oxygen content of shore waters. He found that the amount of oxygen dissolved in sea water at Concarneau fluctuated between 16 and 20 mg. or parts per million, or about 11 to 14 cc. per liter. This large amount is explained by the shallowness of the water along the coast and by the carpet-like colonies of algæ which cover the ground to a great extent in the shallow waters, and in sunlight are able to produce an abundance of oxygen. The best proof of this hypothesis is the fact that the amount of oxygen is greatest during long continued sunshine.

The extensive investigations of Whipple and Parker ('02) on ponds and reservoirs under their care, point to a very close relationship existing between the gas content of the water and the periodic growth of the phytoplankton in these waters. This is especially noted in the spring and autumn maxima of diatoms. The fluctuations in gas content are very much influenced by the movements of the water on a large scale, known as "overturning," at these times.

They say: "The relation of microörganisms to the oxygen content of waters have been investigated, but their relation to dissolved carbonic acid has received scant attention, probably because of the elusive nature of this gas in water and the difficulty of determining its amount. Inasmuch as the algæ and many of the troublesome protozoa are chlorophyll bearing, the relation is one of fundamental importance. All such organisms require both oxygen and carbonic acid in their metabolic processes.

"Nitrates are, indeed, important, but inadequacy of this explanation became manifest when it was observed that some water comparatively low in nitrates, at times supported large growths of algæ. Another reason for the development of the algæ in ground water suggests itself, namely, the presence of carbonic acid. Its occurrence in comparatively large amounts in waters of this class has been shown, and there is good reason to believe that the algæ are influenced by it more than by the nitrates, although the latter are necessary. The consumption of carbonic acid by diatoms has found illustration in several recent instances where *Asterionella* and *Synedra* have developed in ground waters exposed to the light. In the inlet of a certain reservoir where *Asterionella* was abundant, the water contained five to eight parts per million of carbonic acid, while at the outlet it was entirely absent."

Again, while the overturning brings up nitrogen as free ammonia to be oxidized to nitrates when it reaches a supply of oxygen, at the same time a supply of $CO_2$ is carried up by the same current which brings up the diatoms themselves. "It is quite possible that the duration of their growth is determined by the amount of $CO_2$ present, but observations on this point are not convincing."

In another article Whipple ('94) says: "That diatoms need air has already been shown by laboratory experiments; but whether it is the $O_2$ or the $CO_2$ gas of the air has not been determined."

In an earlier article Calkins ('92) shows that there is a maximum of diatoms in April and another in autumn, reaching its climax in December. How much these depend upon gas content and how much on temperature and nitrogenous content is not fully established. Temperature is known to be an important factor.

Horation Parker ('11) found that *Synura* appeared in Lake Chochituate with a temperature of 48° to 50° F. and disappeared when the temperature fell below this. But he says: "Temperature is not the only factor that fostered the organisms at this place (the thermocline), for at higher levels (above the thermocline) there would have been less free carbonic oxide, and at lower ones less oxygen and less sunlight."

Marsh ('03) found *Synedra* in Green Lake, Wis., in nearly every month in the year, but in one winter especially it was flourishing from January to May. This fact, while difficult to reconcile with a part of Parker's theory, confirms his statement in another that the temperature is not the important factor. Fritsch ('07) also believes that the scarcity of diatoms in tropical waters is due to the small amount of dissolved gases in the water, rather than to an unfavorable temperature, though, in general, they prefer colder water.

Studies made by Whipple and Parker ('02) in previous years and also by Baldwin and Whipple ('06) furnish, in a degree, the lacking proofs and serve to confirm the conclusions of Parker as to the relative importance of these dissolved gases for phytoplankton in general. The studies of Marsh ('03) serve, also, in a way, to confirm this idea; for while Whipple and Parker ('02) found diatoms, such as *Synedra, Melosira* and *Cyclotella,* reaching their maximum in spring and summer, as Marsh did in Lake Winnebago, yet he finds it to occur in Green Lake in the depth of winter, for three successive winters. He quotes Voigt, also, as reporting the maximum for *Asterionella* as occurring in winter in Traumer-See and Edeberg-See. This would indicate that temperature is not the deciding factor.

Whipple and Parker ('02), in the studies above cited, describe a large growth of diatoms just below the thermocline, the only place where an abundance of carbonic oxide occurred, while oxygen was to be found at all depths. At this time it was observed that above the thermocline the water was alkaline to phenolphthalein, indicating the presence of a certain amount of normal carbonates in solution. This alkalinity has been more frequently observed in connection with a vigorous growth of blue-green algæ. It is possible that certain organisms under certain conditions have the power of taking carbonic oxide from the hypothetical[1] bicarbonates of alkaline earths. This would accord with the facts noted long ago by one of the authors, that in Massachusetts water supplies, heavy growths of organisms were somewhat more likely to occur in hard waters than in soft waters. This fact has been observed since by a number of others, notably Wesenburg-Lund ('08) and Birge and Juday, to whom reference is made more fully later.

The cause of the sudden appearance of "water-bloom" is but little understood. Whether it is the cause of the changes noted in the alkalinity and gas content of the water, or whether it is the result of these conditions is still a question. Studies have been made to determine its danger to fish, etc., but scarcely anything is known as to the physiological relations to its environment. Steuer ('11) and others have claimed that by the rapid respiration at night of the plants comprising the "water-bloom," the water is robbed of so much dissolved oxygen that fish have been suffocated.

Baldwin and Whipple ('06) more recently report a case in Weequahic Lake, Newark, N. J., of a rapid growth of "water-bloom" followed by the sudden death of large quantities of fish, and attributed by them to the exhaustion of oxygen from the water. They remark that while green algæ are "powerful oxygenators" there is some reason to believe that the blue-green algæ are not so vigorous in this respect. It had been noted for a number of years that oxygen was deficient in reservoirs where the latter organisms predominated.

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[1] Some of the alkaline earth bicarbonates have recently been prepared by Keiser and McMaster, Jour. Am. Chem. Soc. **30** : 1713-17. 1908, and Keiser and Leavitt, Jour. Am. Chem. Soc. **30** : 1717. 1908.

They found that in general the amount of $CO_2$ varied inversely with the amount of oxygen present. When the water became thoroughly mixed, through the action of the wind, there resulted, as a consequence, an even distribution of both the $O_2$ and the $CO_2$ throughout the vertical. When the amount of $CO_2$ was recorded as zero the water reacted alkaline to phenolphthalein, indicating normal carbonates. When the amount of $CO_2$ was recorded as minus it indicated the amount of $CO_2$ abstracted from the bicarbonates dissolved in the water. This power to abstract carbonic acid from the bicarbonates has been noticed, they say, by several observers, but no special attention has been called to the phenomenon. The exhaustion of oxygen from the water they attribute to the decay of the blue-green algæ; for, with the return of oxygen after the decay of the blue-green algæ, the grass-green algæ increased in amount and were believed to be the source of the excess of oxygen, which reached 149% of saturation. Stirring up the water and exposing different parts to the wind tends to reduce the amount of $CO_2$ in the water and at the same time increase the amount of oxygen in the water by aëration. If the circulation is a gentle one it may increase the amount of $CO_2$ by distributing throughout the mass that which is found at the bottom; but if the circulation is violent it may tend to decrease the $CO_2$ by losses into the atmosphere at the surface.

Morren ('41), who made an extensive study of "water-bloom," and especially that form known as red or bloody water, claims that the amount of oxygen and $CO_2$ varies reciprocally with the amount and kind of organisms found in the water; increasing during the day on account of the rapid photosynthesis of the green or reddish organisms, and diminishing in the night (the amount of oxygen) on account of the rapid respiration of the same organisms. Moreover, he claims that the color of these organisms is so much dependent on the oxygen content of the water that he can control the color by varying the supply of oxygen, an increase producing an intenser red and a smaller amount resulting in green.

This explanation might account for the appearance of red *Euglena* in one of the fish ponds in the state hatchery, a little later than the appearance of the bloom on the other ponds. Some claim that such forms may change from holophytic to holozoic and *vice versa,* in a similar manner, along with change of food supply.

Kofoid ('08) reports the red *Euglena* as appearing in the Illinois River, always associated with *Euglena viridis* and, he thinks, possibly only a variety of the latter; but he does not seem to associate it with the variation in oxygen content of the water.

The sudden appearance of a red bloom on Jamaica Pond, near Boston, for a succession of summers, is reported by Mrs. Ellen H. Richards ('01), but it is not associated with any change in the water, except a rise in temperature. It consisted largely, if not wholly, of *Oscillatoria prolifica.*

The report of Birge and Juday ('11) on the dissolved gases of the Wisconsin lakes made its timely appearance after some ten or twelve years of mature study. This served to shed considerable light on the problem in hand.

In these studies they find much importance attaching not only to the free $CO_2$ brought by the convection currents, but likewise, and more especially, to the half-bound $CO_2$ contained in the bicarbonates of calcium and other alkaline earths. In fact these alkaline lakes support the richest algal flora and, consequently, the richest fauna. Lakes containing a large amount of these substances possess, according to them, a source of carbon-dioxide lacking in soft-water lakes and can, therefore, support a large population of plankton. The reduction of bicarbonates to mono-carbonates gives an alkaline reaction to the upper stratum of the lake. These mono-carbonates must take up the carbon-dioxide liberated in the upper water by respiration and decomposition, and more will be absorbed from the air than is possible if free carbon-dioxide is already contained in the water.

Angelstein ('10), working with a number of different kinds of water plants, found that photosynthesis, as shown by the bubble method, was proportional to the concentration of bicarbonates present in the water and that in distilled water, free from carbonates, but saturated with free $CO_2$, the photosynthesis was very slight. Water saturated with air alone gave very slight activity, as shown by the bubbles, but was greatly increased by the addition of a small amount of $KHCO_3$, or $NaHCO_3$. The maximum was reached at about 1% to 1.5% solutions, and above that decreased on account of too high osmotic pressure. Solutions of like osmotic pressure gave like results. Concentrated solutions of calcium bicarbonate always gave a higher oxygen excretion than tap water saturated with free $CO_2$. In natural waters an equilibrium is established between the $CO_2$ pressure of the carbonates in the water and the $CO_2$ pressure of the air.

An abundance of dissolved carbonates, according to Birge and Juday ('11), increases the supply of carbon-dioxide for plant use, both directly and indirectly; directly, by the original stock of half-bound $CO_2$ in the bicarbonates; indirectly, because the mono-carbonates take up more $CO_2$ from the air than would be absorbed without their aid, and also absorb that liberated in the upper water by respiration and decay. Much of this last would escape into the air, especially at night, if not so absorbed.

Morren ('41) confined a part of the water in an aquarium with its contained organisms, under a large balloon flask during the night. Analysis in the morning showed the gases present in a very different proportion from that found in the free water, twice as much $CO_2$ in the flask, showing that there must be a great loss of $CO_2$ from the free water during the night and presumably a like amount of oxygen during the day.

In such hard-water lakes as those above described, Birge and Juday ('11), find the *epilimnion* to be permanently alkaline during the summer, which shows that more $CO_2$ is withdrawn from the bicarbonates than is supplied to them from other sources, and more than is made good at night. The process goes so far that mono-carbonates are precipitated to be redissolved by the free $CO_2$ in the lower water or accumulated in the mud at the bottom.

Davis ('01), in accounting for the origin of the marl in some of the small lakes in Michigan, gives the following:—after pointing out the inadequacy of animal life, evaporation and escape of $CO_2$ on relief of pressure, as causes of the precipitation of the carbonate of lime, the author discusses at length the only alternative hypothesis, namely, that the calcium salts are precipitated through the agency of plant life. In the marly lakes aquatic plants of all kinds become incrusted with calcium carbonate, which is not a true secretion of the plants, for it is purely external, and the same species in other districts are not incrusted.

If the calcium carbonate is in excess in the water, and is held in solution by carbon-dioxide, then the absorption of the latter by the plants causes precipitation of the carbonates upon those parts, stems and leaves abstracting the gas. But if the proportion of calcium carbonate in solution is so small that it would not be deposited even if there were no carbon-dioxide present, the precipitation is explained by the action of the oxygen set free by the living plants, in converting calcium bicarbonate to mono-carbonate:—$CaH_2(CO_3)_2 + O = H_2O + CaCO_3 + CO_2 + O$.

In lakes such as these the vigorous photosynthesis, thus made possible by this increased supply of $CO_2$ in an available condition, is shown by the resulting excretion of oxygen, which often exceeds the point of saturation. This we should expect to occur, since Devaux ('89) has shown that the ratio, $\frac{CO_2}{O_2} = 1$, holds true for submerged aquatics as well as for aërial plants or leaves. Even in winter Knauthe ('99) found a much larger excess of oxygen in some of the ponds which he studied, when they were covered with ice. In one pond he found the surprisingly large amount of 49 cc. of oxygen per liter of water, just under the ice. This was about 515 per cent of the amount required for saturation. A phenomenal increase in oxygen was noted in this pond, the amount at the surface rising from 29.8 cc. on January 16, noon, to 42 cc. on January 17, 12:30 P. M. This period of very high oxygen content lasted three days and was correlated with a very large growth of chlorophyll-bearing organisms. During the daytime, when the organisms were actively excreting oxygen, the water became alkaline as a result of the consumption of carbon-dioxide, presumably taken from the bicarbonates. It would be interesting to know the kind of organisms that were so active at so low a temperature, but this fact is not stated. The mantle of ice prevented the escape of oxygen, which often occurs during a strong wind in summer.

In some of the hard-water lakes studied by Birge and Juday ('11) the alkalinity reached a point where some of the normal calcium carbonate was precipitated out, thus forming a layer in which the combined $CO_2$ showed a pronounced decrease. Alkaline strata have been found in lakes which have very soft water. On the other hand, soft-water lakes, especially with very soft water, present very different conditions. No large supply of free $CO_2$ is readily obtainable, and since only a small quantity of half-bound $CO_2$ will be present in soft water, the total available $CO_2$ will be limited in amount. In general the soft-water lakes of Wisconsin, so far as observed, do not support so large a phytoplankton as the hard-water lakes, and no hard-water lakes have as yet been found which contained so little phytoplankton as many of the soft-water lakes.

Wesenberg-Lund ('08), in his comparative study of the fresh-water lakes of Denmark and Scotland, found that those of Denmark, which are nearly all shallow and usually alkaline, support a rich biota, while the lakes of Scotland, most of which are deep and acid on account of bogs and humic acid in the soil of the drainage basins, are very poor in plankton and submerged aquatics and, consequently, in the ordinary life of fresh-water lakes. The blue-green algæ which form so important an element in the littoral region of the Danish lakes, often covering the stones and depositing on them a layer of greenish lime, are almost non-existent in the corresponding regions in Scotland. As regards the organisms themselves, the Cyanophyceæ play a very much less important part in Scotland than in Denmark, while conversely, the desmids are remarkably well represented in the plankton of the Scottish lakes and but feebly so in the Danish lakes, in common with nearly all central European lakes.

West ('04) notes that "Desmids thrive best in soft water, and they are most numerous in peaty water which has a trace of acid. With few exceptions they do not flourish in water containing carbonates of lime in solution, and no good collection of desmids can be made in a purely limestone district in which the water is hard."

Fritsch ('07) thinks that the scarcity of diatoms in tropical waters, noted especially at Ceylon, is due to the small amount of dissolved gases in the waters, and not to the high temperature, *per se,* except in so far as it influences the amount of dissolved gases. He found them only in aërated waters, *i. e.,* running streams. He did not have data as to the amount of dissolved gases, but assumes *a priori* that a high temperature, 25° C., must of necessity indicate a low gas content, as indicated by figures from Forel's tables. The lowest temperature attained by the lowland waters of Ceylon was 25° C., which in the table corresponds to 5 cc. of oxygen per liter.

| | Temperature | Oxygen | $CO_2$ | |
|---|---|---|---|---|
| Forel | 5° C.<br>20° C. | 7.3 cc.<br>5.7 | 0.6 cc.<br>0.3 | Per liter of water, as found in the waters of Lake Geneva. |
| Roscoe and Lunt | 5° C.<br>20° C. | 8.68<br>6.28 | | Standard.<br>Saturation. |

In the fish-pond, mentioned as an example of temperate zone fresh water, near Bristol, England, Fritsch ('09) finds that diatoms play an important part. The free forms predominate during the winter months, but are rare during the summer months and occasionally absent. The water in this locality and latitude does not become very strongly heated, and hence is not deprived of its dissolved gases, as is the case in the tropics just mentioned. However, the summer is marked by change of filamentous forms, such as *Melosira* and *Fragilaria.* It is believed that diatoms in general prefer colder and better aërated water, although Fritsch mentions the well-known cases of diatoms in hot springs, where they usually assume filamentous forms.

In this connection it is interesting to mention H. W. Clark's ('08) report of *Melosira* in Guatemala, where, he says, "One of the most striking features of the Amatitlan plankton is the abundance of *Melosira,* which is found abundantly in the bottom of nearly all the hauls, and usually makes up the main mass of all the filamentous material. The specimens occur in long rigid filaments."

West ('09) says: "Diatoms are not a feature of the plankton in the Yan Yean reservoir at Victoria, Australia, although *Melosira granulata* occurs abundantly and is never absent from the collections."

"After all," says Fritsch ('07), "We know as good as nothing at present as to the influence of the amount of dissolved oxygen in the water, on the various fresh water algal genera."

As to the desmids, both Fritsch ('07), in Ceylon, and West ('09), in Yan Yean, Australia, remarked the abundance and variety of and tendency toward the filamentous forms. West ('09) says that the most striking feature of the plankton at Yan Yean is the richness of its desmid-flora.

On the other hand, in the fish-pond near Bristol, Fritsch ('09) finds a notable lack of desmids, the number of individuals being small, and likewise the number of genera. This is no doubt due to the fact that water containing carbonated lime, which is known to be unfavorable for an abundant development of desmids, drains into the pond. This fact is also noted in regard to the absence of desmids from the calcareous waters of Kew and the Thames river. The desmids, which generally are so abundant and varied in the temperate waters, were found by Fritsch ('07) to be even more abundant and varied in the waters of Ceylon. The great variety of form, he says, may be only the result of a longer evolution, but the predominance of filamentous forms in tropical waters, which are so rare in temperate waters, indicates an evident adaptation to poorly aërated waters, since they are found to be most abundant in small stagnant ditches and rock pools. Even the species which elsewhere exist as separate simple individuals are here found attempting a filamentous form. This is not merely a local condition, for in the upland pools (altitude 6,000 feet), where conditions are semi-temperate, the filamentous forms are just as rare as in the waters of temperate regions. He has elsewhere suggested that we may again find an explanation for these phenomena in the relative aëration of the waters, *the presence of little dissolved oxygen appearing to encourage filament formation.* This is a suggestion which must be left to experiment for verification, but it certainly would explain the relative abundance of filamentous desmids in the tropical waters. The same theory also will account for filamentous diatoms in the hot springs. In a foot-note it is stated that this theory is based on Senn's observations on colonial Protococcales.

Senn ('99) put *Cœlastrum* cells from pure cultures containing no cœnobia, into culture flasks containing nutrient solution. One flask he stirred up well with air so as to secure good aëration. The other flask was completely exhausted of air by means of a suction pump. After a week samples from the former showed only single cells, no cœnobia having been formed, while samples from the latter showed a number of eight and sixteen-celled cœnobia, perfectly green and with gelatinous conditions well developed.

In another case he put single cells into glass cylinders 12 cm. high, with 0.25% Knop's nutrient solution. Boiling the solution to sterilize it had driven out all the air. In a few weeks this contained cœnobia with gelatinous arms or connections developed. Similar cultures in 2% solutions, instead of 0.25%, gave similar results, showing that the action was not due to concentration of the solution. Moreover, distilled water gave the same results. The depth of the water prevented the diffusion of air to the lower layers of water. From these experiments he concluded that *Cœlastrum* in well aërated water will form free cells, but in stagnant water will form cœnobia. He had gotten similar results from hanging drops of cane-sugar solution. Those which he stirred vigorously and mixed with air produced free cells only, while the unstirred solution formed cœnobia. The stirring had mixed in a considerable amount of air which had remained.

In order to test this further he arranged cultures in Erlenmeyer flasks (0.2% Knop's solution), likewise in open dishes. He boiled them to drive out all the air. After cooling, the flasks were closed and either oxygen or carbon-dioxide let in (manner not stated) to about half (½) pressure, kept air-tight under water, and shaken so as to mix as much as possible and saturate one solution with one respective gas ($O_2$ or $CO_2$). A little of the alga in four-celled colonies from pure cultures was then introduced by means of a curved pipette, and the flasks then closed air-tight with paraffine.

There were eight flasks in all; four with oxygen and four with carbon-dioxide. Two each were placed in the light and two each in the dark. After six days those with oxygen showed many free cells but no cœnobia. In the flasks with carbon-dioxide only a few of the 4-celled cœnobia had changed; but in two weeks many 8-celled cœnobia appeared. Those with $O_2$, left in the dark eight days, showed no change. Those with $CO_2$, left in the dark eight days, were developing daughter cells the same as those left in the light. Similar results were obtained with certain species of *Scenedesmus;* oxygen cultures yielded only single free cells, while cultures with $CO_2$ produced four-celled cœnobia when grown in tall 13 cm. cylinders. Likewise with *Dictyosphærium*; if the culture solution after sterilization was not shaken or aërated, many-celled cœnobia resulted—some even as high as 100-celled; while similar cultures at the same time, but shaken after sterilization, to secure aëration, yielded only single-celled colonies, or a few 4-celled to 16-celled ones. In the latter the cells stood farther apart, while in the former they grew more closely together.

### PRELIMINARY TESTS.

As a preliminary step I made a series of determinations of the dissolved gases, oxygen and free $CO_2$, in the waters of the lagoon and stream of the Garden and in some of the ponds in the city parks, extending from May until the freezing of the ponds in January. The autumn and winter of 1911, up to January, were mild and most favorable for such studies. There was no severe freeze to kill or check the autumn growth, which was especially rich in diatoms. The summer was unusually warm; much of the time ranging from 90° to 100° F., from May to October. But this made only a slight difference in the supply of water in the ponds, which are fed from the City System, and an almost constant level is maintained, except when heavy rains cause a slight temporary rise and overflow at the outlet dam of the lagoon. The hot summer must have had some considerable influence on the temperature of the water, as it nearly always showed the same temperature as the air during the summer and early autumn. The chemical composition of the water fluctuates somewhat with the heavy rains, but aside from this it must be the same as that of the Mississippi River, from which it comes.

While the chemical value of the water as a culture medium for algæ is not materially changed by the process of purification at the city settling plant, yet there are slight changes that would have to be taken into account where chemical indicators are involved. Miss Hayden ('10), in her recent study of the algæ in these ponds, makes a general statement on authority, to this effect. The annual report of the Water Commission for 1906, the last available, shows that the process removes nearly all of the bicarbonate of calcium and delivers the water with quite a strong degree of alkalinity. This was a point with which I was later to reckon.

### INVESTIGATIONS—FIELD.

The accompanying table of oxygen and carbon-dioxide content of the water in the lagoon shows an increase of oxygen from 3.3 cc. per liter, June 23, to the saturation point of 7.80 on July 20, when the "water-bloom," consisting mostly of blue-greens, chiefly *Anabaena circinalis, Clathrocystis aeruginosa* and *Coelosphaerium Kuetzigianum,* appeared. At the same time the water had changed from neutral through slightly alkaline to 5 cc. alkalinity during the same time, and on up to 7 cc. alkalinity a month later, indicating the complete absence of free $CO_2$. This condition remained for over a month, or until September 12, when the rains and cloudy days restored neutrality and reduced the amount of oxygen in solution, partly by the presence of decaying organic matter.

From this time on to middle or late December the amount of oxygen decreased and the $CO_2$ increased, the alkalinity disappearing and the water showing a large amount of free carbon-dioxide. This does not mean that these are mutual opposites acting like a mechanical couple. The increase in one is not always proportional to the decrease in the other, and vice versa, though in general that is true. There may be either an increase or a decrease in the amount of oxygen without any change in the alkalinity or amount of $CO_2$, and vice versa; but in general, the conditions which favor the increase of one cause a decrease in the other; in other words, it seems evident that the water is rendered neutral and later alkaline by the withdrawal first of free $CO_2$ and then of the half-bound $CO_2$ from the bicarbonates by the rapid growth and active photosynthesis of plankton algæ and shore aquatics which excrete an excess of oxygen. If the water is not disturbed by wind this may go on to supersaturation, which was really the case July 20 to 30, August 21 and September 12. Saturation for that temperature, 27° C., according to the saturation tables of Roscoe and Lunt, is only 5.6 cc. per liter, while my figures for these dates show 7 cc., or 130%, of saturation. Thus while we should expect a decrease in both oxygen and carbon-dioxide with rise of temperature we find, instead, an increase of oxygen, due evidently to plant growth and activity, which diminishes the carbon-dioxide far below the saturation capacity or absorption coefficient.

It will be further noticed that these fluctuations correlate with cloudiness and sunshine. The first figure, low, with 3.3 cc. of oxygen, June 23, is after rain and cloudy weather, while the high figure, 7.34 cc., July 30, is after a long space of clear weather and the appearance of the "water-bloom," which seems to have been the important agent in producing the increased amount of oxygen.

The lowest figure shown for oxygen, August 25, is after two days of cloudy weather, though the accompanying fall in temperature would increase the water's capacity. Again, on August 28, we have combined rain, cloudiness and wind. The wind diminishes the oxygen content by stirring the water and making supersaturation impossible. September 5, these conditions are augmented by a heavy rain (2.5 inches), which reduced the oxygen figure to 3.44 cc. The heavy rain left the lagoon so clouded as to interfere with photosynthesis, and the organic matter carried in by surface drainage, including some sewage, would greatly reduce the oxygen. It is worthy of note that the decrease was not so great in the upper basin, or Fountain Pool, which was less affected by surface drainage (figures not shown in the table).

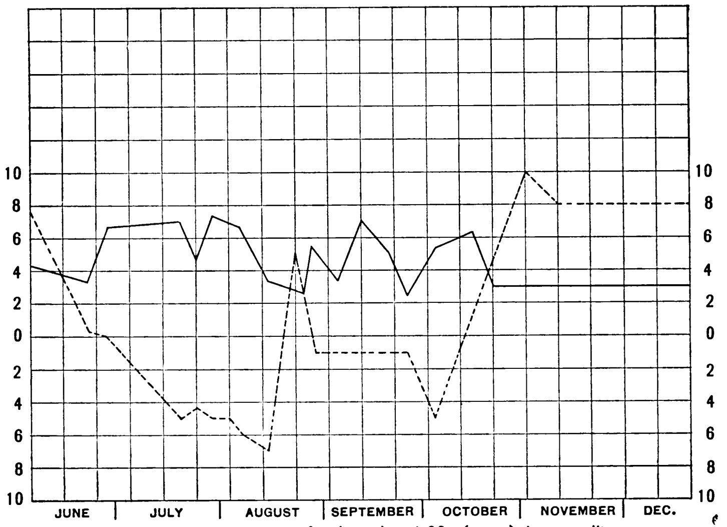
At this time, also, the "bloom" sank out of sight for a time, apparently on account of the cooling of the water, or possibly on account of a change in the specific gravity of the organisms composing the "bloom," so that photosynthesis probably was almost nil in the water darkened by the large amount of matter in suspension. The same conditions were repeated September 26, when the oxygen figure reached its minimum of 2.32 cc. These data, small in number, selected from a much larger number, are presented, not on account of their agreement with any particular theory, but because they are representative. Those not given are omitted simply because they are not significant.

One thing might be misleading in the column on cloudiness, as long lists of clear days have been omitted during which there was no change. This brings a disproportionate number of cloudy days into too close a sequence.

| DATE | Temperature Centigrade | Condition of Sky | Oxygen cc. per Liter | Per cent of Saturation | $CO_2$ cc. per Liter. or Alk. ( — ) | REMARKS, LOCATIONS, ETC. |
|---|---|---|---|---|---|---|
| June 5... | 29° | Clear | 4.600 | 84 | 7.7 | In tap water. |
| June 23... | 29° | Cloudy | 3.360 | 61 | Trace | Lagoon at bridge, air very cool. |
| June 28... | 26° | Clear | 6.55 | 116 | Slightly Alkaline | After rains. |
| July 20... | 26° | Clear | 7.10 | 125 | —5.00 | Fountain pool. |
| July 25... | 27° | Clear | 4.53 | 81 | Alkaline | Lagoon at bridge. |
| July 30... | 27° | Clear | 7.34 | 131 | Alkaline | "Bloom" appeared. |
| Aug. 7... | 27° | Clear | 6.50 | 116 | —5.00 | Fountain pool. |
| Aug. 15... | 26° | Clear | 3.55 | 61 | —6.00 | Smoky, hazy. |
| Aug. 21... | 26° | Cloudy | 3.08 | 54 | —7.00 | Lagoon at bridge after two days of cloudy weather. |
| Aug. 25... | 22° | Cloudy | 2.60 | 43 | + 5.00 | |
| Aug. 28... | 22° | Cloudy | 5.54 | 92 | Alkaline | Shower, windy, 2½ inches rain fell previous night; water over dam. |
| Sept. 5... | 24° | Cloudy | 3.44 | 58 | Alkaline | |
| Sept. 12... | 27° | Clear | 7.37 | 131 | Alkaline | Sultry day. |
| Sept. 21... | Air 18°<br>Water 20° | Cloudy | 5.53 | 89 | Neut. | |
| Sept. 26... | 22° | Cloudy | 2.32 | 39 | Neut. | Water muddy after heavy rains. |
| Oct. 2.... | 20° | Very Cldy. | 5.52 | 88 | Neut. | After 2 days' rain |
| Oct. 14.... | 22° | Clear | 6.193 | 102 | 5.00 | |
| Nov. 21... | Air 13°<br>Water 4° | Clear | 2.92 | 32 | 5.00 | Clear and frosty. |
| Dec. 1... | Air 10°<br>Water 3° | Clear | 3.00 | 33 | 10.00 | First ice on lagoon. |
| Dec. 18... | Air 0°<br>Water 0° | Clear | 3.00 | 31 | 10.00 | Thick sheet of ice. |
| Dec. 27... | Air 2°<br>Water 0° | Cloudy | 3.50 | 38 | 8.00 | |
| Jan. 20.... | Air 4°<br>Water 0° | Clear, like April | 3.76 | 41 | 8.00 | Samples from under thick ice. |
| Feb. 15... | Air 12°<br>Water 4° | Hazy | 4.18 | 47 | 4.25 | Thaw after long cold spell, thick ice. |

The above figures and the interpretation of them agrees, in the main, with those of Morren ('41) already mentioned. His were given for every day for the entire year. It is hoped that an extension of the above data may serve to confirm the interpretations and conclusions already reached. The later dates are less significant. The "bloom" disappeared with the frost of early November, and there was a decrease in the oxygen content of the water, reaching a minimum again November 21, when the lagoon was almost clear of vegetation. This, with the autumn rains bringing in drainage water, kept the oxygen figure down, while the alkalinity gives place to neutrality for a time, to be followed by an increasing amount of free $CO_2$, reaching a maximum in December. The

GRAPH I.

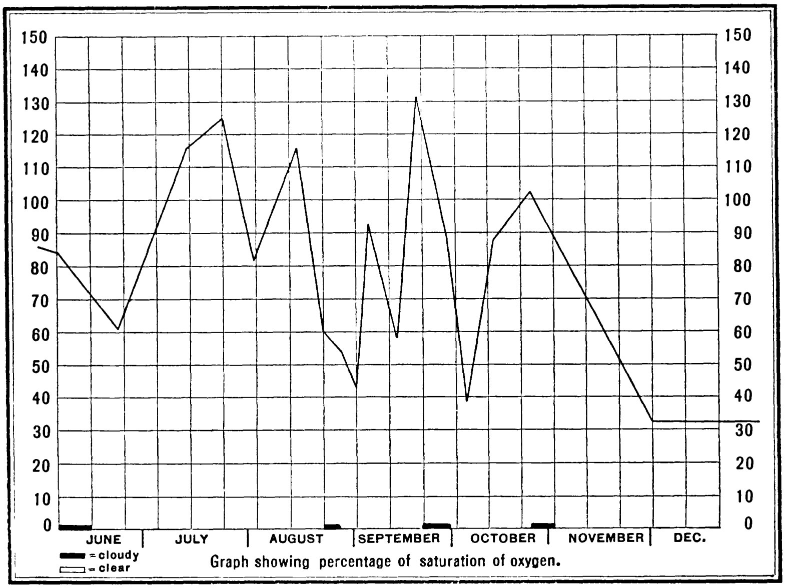


Graph showing the amount of $O_2$ (——) and $CO_2$ (----) in cc per liter.

decay of organic matter washed from the dead leaves of a late autumn and the gradually decaying green algæ, together with the decay of the blue-greens of the water-bloom, would materially increase the amount of $CO_2$ and consume the oxygen produced by the fall growth of diatoms and grass-greens. After the thick ice sheet was formed about mid-December there was but little change. The oxygen and carbon-dioxide figures both seek the medium and remain there quite constantly. There is but little live vegetation under the ice, and what is there cannot be very active. The same must be true of any animal life that may be present.

The column showing the per cent of saturation and oxygen shows a more striking contrast than the column of cc., which strongly indicates that it must be due to biological rather than to meteorological influences. This is more strikingly shown in the graph showing per cent. of saturation.

GRAPH II.



Graph showing percentage of saturation of oxygen.

## EXPERIMENTATION IN LABORATORY— GENERAL.

In attempting to arrange a set of experiments under control conditions to show on a small scale the effect of an increase or decrease of the two gases, oxygen and $CO_2$, a plan was hit upon of driving these gases, as generated, through a series of flasks partly filled with tap water or pond water, and containing the algæ most available or most likely to respond.

Accordingly, small Erlenmeyer flasks were fitted with two-holed stoppers. Into one hole was a leading-in tube of glass reaching to the bottom. From the other hole was a leading-out tube of glass going to another flask like the first, and reaching nearly to the bottom of the same; and so on through the series. In the same manner the gas driven in at one end could, with little pressure, be forced through the entire series and leave them all saturated and filled with the gas.

Into each flask was put 50 cc. of tap water and a small amount of selected alga. Each flask contained only one species, although there was no attempt at pure cultures nor to rigidly exclude all others.

The generator was connected and the gas passed in to as near saturation as possible; then it was left in diffuse light at ordinary room temperatures, 18° to 20° C. They were left so for about a week when they were again charged with gas. There were two series; one to be treated with oxygen and the other with carbon-dioxide. A third series for control was not to be treated with either.

In order to secure purity of gases and prevent any poisonous or ill effect from that source, a wash-bottle was placed in series between the generator and the culture flasks. The wash-bottle for the $CO_2$ generator contained a solution of sodium bicarbonate to arrest any HCl gas that might escape from the generator, in which hydrochloric acid and lumps of marble were used. The gas was tested until it gave no precipitate with $AgNO_3$. Preliminary trials showed that this was necessary, as some HCl came over when the wash-bottle was not used, as shown by the above test and the blackening of the plants in the first flasks.

For the oxygen generator the wash-bottle contained only water, as there was no volatile substance nor anything injurious except a little NaOH and a compound of copper in the "oxone," a sodium peroxide compound used for the generation of the oxygen.

The generators were so regulated that the gas came off in a moderately slow stream of bubbles and continued long enough to insure complete saturation. The end flask contained nothing but water and served to prevent the entrance of air or atmospheric gases.

November 14, the following were used and placed in the order named: *Elodea, Pithophora, Cladophora, Spirogyra* and *Anabaena.*

November 21, *Elodea* in oxygen seemed to be dead, while that in the $CO_2$ seemed brighter than the check. All were renewed and continued as before.

November 26, *Cladophora* in $CO_2$ was dead and disintegrated.

*Cladophora* in $O_2$ was much like check; others unchanged. The above results gave little promise of any conclusions further than to indicate the possibility of continuing an experiment on this plan and that different plants showed a difference in response and endurance. But other factors would likely enter into such treatment, as light, temperature and dissolved salts.

December 7, new material was arranged and continued to December 24, when the conditions were as follows:—

| Condition Dec. 24 | Large Hydrodictyon | Small Hydrodictyon | Cladophora | Stigeoclonium | Pithophora | Elodea |
|---|---|---|---|---|---|---|
| Check.... | Bright | Spoiled | Unchanged | Dead | Green | Dead |
| In $CO_2$... | Fair | Improved | " | " | " | Green |
| In $O_2$..... | Good | Very Bright | " | " | " | Nearly Dead |

A large amount of *Elodea* was kept in jars in various conditions as to light, etc., and it all did well, so the material was not at fault. *Stigeoclonium* did not do well under any conditions.

Not much change occurred in any of the cultures, except that *Clathrocystis* (from which important results were expected) soon died in all the series as it had done under various kinds of treatment throughout the summer. One large jar, after the excessively hot weather was over, kept in moderate light and temperature (about 20° C.), lived for over a month, but died, eventually, without any apparent cause.

In the above table it will be seen that *Hydrodictyon* in oxygen became much brighter than the check or that in $CO_2$. This was true of all the others in oxygen, though in less degree. This fact, though needing further confirmation, agrees with that of Senn ('99), obtained with *Cœlastrum.* He placed cultures of *Cœlastrum reticulatum* in culture solution (0.2% Knop's solution) in Erlenmeyer flasks, one set being supplied with oxygen and the other with carbondioxide somewhat as my cultures were. He placed some in the dark and some in diffuse light. After six days the flasks were opened and investigated. He says: "Schon dem blossen Auge boten die Sauerstoffculturen einen ganz anderen Anblick als diejenigen mit Kohlensäuere. Während man bei den letzteren mit Mühe am Boden des Fläschens einen grünen Schimmer entdecken konnte, zeigten die Sauerstoffculturen einen deutlichen, prächtig frisch grünen Bodenüberzug. Unter dem Mikroskop stellte es sich nun heraus dass die mit Sauerstoff cultivirten Algen zahlreiche einzelne, prächtig grüne aussehende Zellen gebildet hatten; dieses frisch grüne Aussehen hatte seinen Grund darin, dass die Zellen ihre Stärke völlig verathmet hatten."

Still further, and, perhaps, more remarkable, he produced bright green chlorophyll in yellow resting cells of the same plant in ten days, by means of a culture solution saturated with oxygen, while similar cultures supplied with $CO_2$, instead of oxygen, produced only a reddish-yellow color. These cultures, with $CO_2$, were placed in the light. Of those containing oxygen (four in all) two were placed in the light and the other two were left in the dark. Oxygen cultures in distilled water remained entirely red, showing that culture solution (food) was necessary for the formation of chlorophyll.

With *Cœlastrum microporum* he was less successful, especially in cultures exhausted with the air-pump. The cultures with oxygen showed at the end of the week almost exclusively bright green cells, scarcely colored by iodine. Those left in the dark had increased little or none, were pale green and turned dark brown when treated with iodine. The first series of $CO_2$ cultures nearly all died on account of the strong concentration of the gas used. A second experiment with a weaker concentration of $CO_2$, after five days, showed chiefly cœnobia with spherical cells which colored intense brown with iodine. In general, the species *C. microporum* was less resistant and more likely to perish. It was also more apt to form single free cells and less so to form cœnobia than in the case of *Cœlastrum reticulatum.*

Among my cultures was a flask containing, among other things, *Cœlastrum microporum* in Knop's solution, which had been standing quietly on a laboratory table for six months. On examination it was found to contain many cœnobia of eight, sixteen, and some of thirty-two cells; some of these were beginning to form daughter-cells, or young cœnobia. No such large colonies were to be found when the original material was taken from the pond in September. Although the culture fluid was shallow in the flask, about 1 cm. deep, and the flask plugged only with cotton, the conditions were essentially those of stagnant water, and agree closely with those employed by Senn ('99) in some of his work, though it would require duplicates and extensions to justify any final conclusions.

Some *Cladophora* placed in a glass jar with a loose glass lid and nearly filled with tap water (stagnant condition) showed a much more branched condition after a number of weeks than the original specimen taken from flowing water, or any of the specimens found in free open water, although the water, light and other conditions, aside from aëration, were the same. The new cells, moreover, were shorter and thicker than in the older parts of the original specimen, and the cells had broad, thickened, or inflated ends, that showed a branched appearance or an abnormal tendency toward branching. In the older cells the length is usually ten or more times the diameter; but in these newer cells, and especially the end ones, the length is scarcely three or four times the diameter.

Fritsch ('09), in noting the seasonal changes of *Cladophora* in Abbot's Pool, near Bristol, England, describes the inflated type as characteristic of winter, and the much-branched type as characteristic of winter, late spring and summer; so it is a little difficult to interpret this or relate it to any reported conditions.

A similar tendency was shown by one culture of *Hydrodictyon.* In this the cells had all acquired a zigzag form, with inflated, club-shaped ends. They soon developed spores, or gametes, and disintegrated, with no further development. This was in an open jar of tap water which had evaporated to about one-eighth of its original volume.

Freund ('08), in an extensive article on the influence of environment on vegetative reproduction, says: "More probable, though less clear, is the influence of the oxygen content of water. The assertion that zoospore production can be induced merely by treatment with oxygen, as stated by Waltz, has been disproved by Klebs for *Vaucheria repens* and *V. clavata.* Simply by increasing the oxygen content in the surrounding medium, so far as our present knowledge goes, or may allow us to judge, no alga can be aroused to zoospore formation. On the other hand, has it not been accomplished by the removal of oxygen? Klebs made a study of *Vaucheria* and *Hydrodictyon,* which indicates that many algæ *(Vaucheria repens, V. clavata, Hydrodictyon,* and *Oedogonium diplandrum; Stigeoclonium tenue, Draparnaldia* and *Ulothrix zonata)* can be induced to form zoospores if they be removed from flowing water to stagnant water, which indicates that reduction of oxygen in the medium is, in this case, the regulating responsible factor. The possibility of the influence of a reduction in oxygen is not excluded when algæ like *Botrydium* and *Vaucheria,* growing in moist air, produce zoospores by moistening with water. Naturally, in this case, other influences come in, such as the absorption of water, etc., which were not previously distinguishable."

None of the cultures, so far as I could discover, showed any tendency to zoospore formation in response to the stimulus of oxygen or $CO_2$. The tendency toward periodic spore production seems to be too deep-seated in heredity to be easily overthrown by any artificial external stimulus. The observations of Fritsch ('07), Benecke ('08), Copeland ('09) and Danforth ('10), lend great weight to this idea, as do also the studies of Williams ('05) on the Dictyotaceæ. However, Benecke and Fritsch concede that if we could fully imitate the conditions found in nature we might get the same results regardless of season.

### EXPERIMENTATION IN LABORATORY—SPECIAL.

In order to test more fully what I had noticed, as to the water becoming alkaline by the growth of algæ, I arranged several series of cultures with and without carbonates of various kinds.

November 24. Test-tubes were filled with distilled water and boiled to drive out any $CO_2$ that might be present; then they were stoppered and allowed to cool. When cool, small quantities of the following plants were put in: *Hydrodictyon, Oedogonium, Elodea* and *Spirogyra.* The tubes were stoppered as tightly as possible and placed near an east window. In another set of test-tubes with distilled water as in the others, instead of boiling, a little powdered calcium carbonate was used, which was intended to absorb all the free carbon-dioxide. These were supplied with plants from the same source as the others, stoppered in the same manner and placed alongside the others, near a window.

December 24. At the end of a month the *Oedogonium* and *Spirogyra* in the tubes of boiled water were dead and black, attacked by moulds and beginning to putrefy. The *Hydrodictyon,* though still green and apparently alive, showed no oxygen bubbles or other sign of activity, like that of the check material. The *Elodea,* likewise, was green, but dormant. All the tubes containing lime carbonate were bright and active, as shown by an abundance of oxygen bubbles. Since all the tubes were tightly stoppered to exclude the air, the only source of $CO_2$ for photosynthesis was that of the bicarbonates, which showed that the plants named could utilize the $CO_2$ from this source.

The above-named plants had been kept in stock in open jars or flasks supplied with tap water, which contains considerable lime in solution and is slightly alkaline to phenolphthalein. This usually loses its color when plants are first introduced, but becomes deep red again after a day or two in bright sunlight or passing $CO_2$ through the water. A period of darkness had the same effect of destroying or withdrawing the color. But the color returned again within a few hours of bright sunlight, and the process was repeated with the alternation of darkness and light.

This I attributed to the action of photosynthesis during the day, withdrawing $CO_2$ from the water, thus changing the solution from a weakly acid one to a neutral and alkaline one, causing the red color; and respiration by night or the balance of respiration over photosynthesis on cloudy days restoring the lost $CO_2$ and again rendering it neutral, or acid and colorless.

This I believed at the time, to be an original discovery and interpretation of my own, but on investigation found that, among a number of others, Hassak ('88) had given the same interpretation, backed by proofs at considerable length. To this, however, Loew ('93) found objections, and claimed that the coloring was due, not to a normal carbonate of lime, as explained by Hassak, but to the excretion of a colloidal compound of calcium, which is alkaline to phenolphthalein. Hassak, in proof of his stand-point, states that sodium bicarbonate gave the same results as calcium bicarbonate, and he believed that other carbonates would do the same if he only had the opportunity to try them.

Hassak used 0.1% and 0.2% solutions in his experiments. Accordingly I prepared 0.1% and 0.2% solutions of sodium bicarbonate, using melted snow, as it was necessary for the experiment that the water be free only from carbonates.

January 9. Into these were put *Elodea, Pithophora, Cladophora, Oedogonium, Hydrodictyon* and *Spirogyra.* Another set of blanks was used as a control, containing only water and plants, and another with nothing but water. To each tube were added three drops of phenolphthalein (1%

in 50% alcohol). The tubes were boiled, cooled, and the plants introduced. They were then tightly stoppered and placed in the light of a south window.

February 6. After nearly a month the blanks remained colorless. This showed that the alkalinity did not come from the excretions of the plants themselves, as it had been claimed by Loew. All the tubes containing both plants and sodium bicarbonate turned deep red on bright days, and faded during the night or cloudy days, which were numerous enough at that time. *Spirogyra,* alone, gave little or no color, owing, apparently, to its poor condition. The intensity of the color varied with the plants in the order named, *Elodea* giving the deepest, and *Spirogyra* the faintest. There seemed to be little, if any, difference between the 0.1% and 0.2% solutions. The results were in all essential respects similar to those in the solutions of lime-carbonate.

Cultures prepared in the same manner, but using potassium bicarbonate, gave even more decided results. This is all the more significant since cultures with potassium nitrate, instead of carbonate, failed to give any color, as Loew claimed, but soon died, instead, and became infested with moulds. In so far this indicated that the important thing is the carbonate in the production of alkalinity.

February 2 to March 2. A similar set of experiments, using ammonium-carbonate, resulted only in the death of the plants. This was likely due to the toxic effect of the ammonium which, according to the experiments of Livingston ('05), is five times as toxic as the above cations in the same concentration, and also Andrews ('05) showed that hairs of *Tradescantia virginica* were killed if left longer than one minute in a one per cent solution of ammonium-carbonate, and were affected adversely in a 0.5% or 0.25% solution of the same.

Another set of cultures like the above, but containing manganese carbonate, although run for nearly a month, gave no color. This is probably due to the low degree of solubility and dissociation of this compound.

One of the strongest arguments against the theory of Loew is the fact that the first time this color reaction was noticed by me was in a small flask of pond water left in a south window during the very bright and very hot days of the summer of 1911. The color appeared faintly at first and increased from day to day until the cause was searched out.

At first the flask seemed to contain nothing but the water, no plant material being visible; but closer investigation showed that the change in color was due to a few microscopic algæ, chiefly Desmids, *Pediastrum* and the like, which, as before mentioned, have little tolerance for calcareous waters, and do not, as a rule, have lime deposited on their surfaces. These algæ developed mostly as a result of multiplication in the flask.

### INCREASED AND REDUCED $CO_2$ AND $O_2$ PRESSURE.

In order to test the conduct of higher pressures of $CO_2$ upon the dissolved carbonates and contained algæ, the following experiments were prepared:—

A. February 10. A half-pint milk bottle was half-filled with distilled water; three small crystals of $KHCO_3$ were added and three drops of phenolphthalein, as in former experiments; this gave no color. Into this were put about 5 grams of *Pithophora.* The bottle was then stoppered with a rubber stopper, attached to a suction pump and exhausted to one-half vacuum, then refilled to atmospheric pressure with $CO_2$ from the attached generator. The jar was then closed air-tight and placed in the light of a south window. In a few days the color appeared, indicating photosynthesis and the reduction of bicarbonate to normal carbonate. The color faded during the night and cloudy days, reappeared on bright days, more especially when in the bright sunlight, just as the previous cases with open vessels and tap water.

B. A control jar like "A," except that tap water alone was used, without $KHCO_3$. This went through the usual color changes.

February 24. Jar "A" was opened and a lighted match applied. The flame flared up more brightly, showing that the $CO_2$ had been absorbed and that oxygen had been thrown off instead. During the interval masses of bubbles appeared in this jar just as in the control and in previous experiments, evidently bubbles of oxygen, as shown by the lighted match, and showing that vigorous photosynthesis had been going on in the jar, making use of the excess $CO_2$.

March 1. This jar, "B," was again exhausted and refilled as before, causing the color to disappear rapidly as the $CO_2$ was absorbed, thus changing the normal carbonate to bicarbonate. After a few days the color reappeared, bubbles formed, showing continued photosynthesis as before.

March 14. A lighted match indicated, as before, that the $CO_2$ had been absorbed and replaced by oxygen.

As further control for comparison, a jar half-full of tap water, which was alkaline to phenolphthalein, was half exhausted and refilled to atmospheric pressure with $CO_2$, which quickly dispelled the color just as when the plants were present. But the color did not reappear when placed in sunlight, as it did when the plants were present. This seemed to leave no further doubt that the alkalinity and color changes were due to the photosynthetic activity of the plants, thus appropriating the $CO_2$.

February 27. Another experiment like the above was set up in which *Hydrodictyon* was substituted for *Pithophora,* using only tap water, and the same amount of $CO_2$ as before. The color faded in about 20 minutes and did not reappear again when placed, like the other, in the south window for over two weeks.

March 16. The *Hydrodictyon* seemed colorless and dead.

March 16. An attempt to revive with oxygen was a failure. Apparently this concentration of $CO_2$ is too great for *Hydrodictyon,* which is not so resistant as *Pithophora.*

Schützenberger ('73), on the basis of some work with *Elodea,* states that for water plants 5% to 10% of complete saturation with $CO_2$ is the optimum. I have not found any account of work done on algæ with high per cents of carbondioxide.

## APPARATUS AND MATERIALS.

In all experiments in which measured amounts of gases were employed, or definite pressures of gases were desired, a Bunsen suction pump with manometer was used, and gas generators were attached in the manner described by Wesbrook ('05), or somewhat similar to that described and used by Chapin ('02) in his studies on the influence of $CO_2$ on plant growth.

The method of collecting and determining the amounts of oxygen in water was that described and used by Whipple and Parker ('02) in their studies. The method is commonly known as Winkler's method. It is more fully described by Birge and Juday ('11).

Another method, based upon the reducing power of ferrous sulphate, was also used as a check and as an alternative, many determinations being made by both methods.

Samples were usually taken and determined in duplicates, and, in case of doubt, were many times repeated. The purest of chemicals obtainable were used throughout.

## SUMMARY.

1. There is an intimate and mutual relation between the algæ and submerged aquatics in a body of water and the gases dissolved in that water. They fluctuate together.

2. Air, or its constituents, oxygen and $CO_2$, are as essential to water plants as water is to land plants, and equally difficult to secure.

Warm and stagnant water is poorer in these essentials than colder water gently agitated by wind or currents.

4. Currents are especially beneficial to attached plants by renewing or removing these gases.

5. Some species demand more aëration than others. Some species are more tolerant of stagnant waters than others.

6. Filamentous forms with large cells and thin outer walls are best adapted to stagnant waters. Such forms predominate in warm, tropical fresh waters, which are poorly aërated.

The photosynthesis of rapidly-growing algæ and aquatic plants in a body of water may diminish or deplete the supply of $CO_2$ and increase the oxygen content beyond saturation.

8. In the absence of free $CO_2$ the plants may utilize the half-bound $CO_2$ of the dissolved bicarbonates, chiefly those of calcium and magnesium.

9. The process of photosynthesis may be so vigorous as to exhaust the half-bound $CO_2$ and render the water alkaline. By respiration and absorption of $CO_2$ from the air more bicarbonates may be formed. This serves as a mechanism for the conservation of $CO_2$.

10. Waters rich in lime-carbonates are also rich in vegetation. Bog waters, containing humic acids, and, consequently, poor in carbonates of lime, are known to be poor in vegetation.

11. Stagnant water, on account of the large amount of $CO_2$ and the small amount of oxygen, favors the formation of colonies and filaments rather than of free individual cells.

12. Colonies and filamentous forms may be produced artificially with some plants, by increasing the amount of $CO_2$ or diminishing the amount of oxygen in the culture solutions.

13. Narrow, much-branched filaments are adapted to and produced by poorly aërated waters.

14. Aëration, or abundance of oxygen, apparently favors the formation of chlorophyll; and algæ are brighter green when well aërated.

15. The periodicity of spore formation is not readily influenced by aëration or gas content of the water. It seems to be more a matter of heredity.

## BIBLIOGRAPHY.

Andrews, F. M. ('05). The effect of gases on nuclear division. Ann. Bot. **19**: 522-530. 1905.

Angelstein, Udo. ('10). Ueber die Kohlensäureassimilation submersed Wasserpflanzen in Bikarbonat-und Karbonatlösungen. Beiträge z. Biol. d. Pflanzen **10**: 87-117. 1910.

Baldwin, H. B. and Whipple, G. C. ('06). Observed relations between carbonic acid and algæ growths in Weequahic Lake, N. J. Rept. Am. Pub. Health. **32**: 167-182. 1906.

Benecke, W. ('08). Ueber die Ursachen der Periodicität in Auftreten der Algen auf Grund von Versuchen über Bedingungen der Zygotenbildung bei *Spirogyra communis.* Internat. Rev. d. Hydrobiol. u. Hydrograph. **1**: 533-552. 1908.

Birge, E. A. and Juday, C. ('11). The inland lakes of Wisconsin; dissolved gases and their biological significance. Wisconsin Survey. Bull. 22. 1911. (Sci. Series 7.)

Blackman, F. F. ('04). Chromatic adaptation. New Phytologist **3**: 237-242. 1904.

Brown, H. E. ('08). Algal periodicity in ponds and streams. Bull. Tor. Bot. Club. **35**: 223-248. 1908.

Calkins, G. N. ('92). Seasonal distribution of microscopic organisms. Mass. State Board of Health **1892**: 383-390.

Chapin, P. ('02). Einfluss der Kohlensäure auf das Wachsthum. Flora **91**: 348-379. 1902.

Clark, H. Walton. ('08). Holophytic plankton of Lake Atitlan and Amatitlan. Proc. Biol. Soc. Wash. **21**: 91-106. 1908.

Copeland, W. F. ('09). Periodicity in *Spirogyra.* Bot. Gaz. **47**: 9-25. 1909.

Danforth, C. H. ('10). Periodicity in *Spirogyra.* Ann. Rept. Mo. Bot. Garden **21**: 49-59. 1910.

Darwin, Fr. R. and Pertz, Miss ('96). On the effect of water currents on the assimilation of aquatic plants. Proc. Cambridge Phil. Soc. **9**: 76-90. 1896.

Davis, C. A. ('01). A contribution to the natural history of marl. Jour. of Geol. **8**: 485-497. 1901.

Devaux, H. ('89). Mechanisme des exchanges gazeux des plantes aquatiques submergées. Ann. d. Sc. Nat. Bot. VII. **9**: 35-179. 1889.

Disato, D. A. ('08). Recherches oceanographiques. Revue Hydrobiol. **1**: 280-281. 1908.

Freund, Hans. ('08). Neue Beobachtungen über die Wirkung der Ausenwelt auf die ungeschlechtliche Fortpflanzung der Algen. Flora. **98**: 41-100. 1908.

Fritsch, F. E. and Ritch, Florence. ('07). Studies on the occurrence and reproduction of British fresh-water algæ in nature. Ann. Bot. **21**: 423-436. 1907.

——. ('09). Studies on the occurrence and reproduction of algæ in nature. Proc. Bristol Natl's. Soc. **2**: 27-54. 1909.

Gaidukov, N. ('04). Die Farbe der Algen und des Wassers. Hedwigia **43**: 96-118. 1904.

Hassak, Carl. ('88). Ueber das Verhältniss von Pflanzen zu Bicarbonaten und über Kalkincrustation. Unters. aus d. Bot. Inst. zu Tüb. **2**: 469-475. 1886-88.

Hayden, Ada. ('10). The algal flora of Missouri Botanical Garden. Ann. Rept. Mo. Bot. Gard. **21**: 25-47. 1910.

Knauthe, K. ('98). Der Kreislauf der Gase in unseren Gewässern. Biol. Centr. **18**: 783-805. 1898.

——. ('99). Beobachtungen über den Gasgehalt im Winter. Biol. Centr. **19**: 783-789. 1899.

Kofoid, C. A. ('08). Plankton of the Illinois River. Ill. Lab. Nat. Hist. Bull. 13. 1908.

Livingston, B. E. ('05). Chemical stimulation of an alga. Bull. Torr. Bot. Club **22**: 1-54. 1905.

Loew, O. ('93). Worauf beruht die alkalische Reaction welche bei Assimilationsthätigkeit von Wasserpflanzen beobachtet wird. Flora. **67**: 419-422. 1893.

Marsh, C. D. ('03). The plankton of Lake Winnebago and Green Lake. Wis. Survey Bull. 12: 1903. (Sci. Series 3.)

Morren, A. and C. ('41). Recherches sur la rubifaction des eaux et leur oxygenation par les animalcules et les algues. Bruxelles. 1841.

Pfeffer, W. ('00). The physiology of plants. Oxford. 1900.

Parker, H. N. ('11). *Synura* in Lake Cochituate, Mass. Journ. Amer. Micr. Soc. **30**: 141-144. 1911.

Richards, Mrs. E. J. and Hyams, Isabel F. ('01). Notes on *Oscillatoria prolifica* in Jamaica Pond. Tech. Quar. **14**: 302-310. 1901.

Schützenberger. ('73). Sur la respiration des vegetaux aquatiques immerges. Compt. Rend. **77**: 272. 1873.

Senn, Gustav. ('99). Ueber einige coloniebildende einzellige Algen. Bot. Zeit. **57**: 39-104. 1899.

Steuer, A. ('11). Leitfaden der Planktonkunde. 1911.

Wesbrook, F. F. ('05). Laboratory methods and devices. Amer. Pub. Health. **30**: 304-324. 1905.

Wesenberg-Lund, ('08). Comparison of Scottish with Danish lakes. Revue Hydrobiol. **1**: 184-187. 1908.

West, G. S. ('04). British fresh water algæ. Cambridge. 147. 1904.

——. ('09). The algæ of the Yan Tean reservoir, Victoria, Australia. Journ. Linn. Soc. **39**: 1-88. 1909.

Whipple, G. C. ('96). Some experiments on the growth of diatoms. Tech. Quar. **9**: 145. 1896.

——. ('94). Some observations on the growth of diatoms in surface waters. Tech. Quar. **7**: 214-231. 1894.

Whipple, G. C. and Parker, H. N. ('02). On the amount of oxygen and carbonic acid in natural waters and the effect of these gases upon the occurrence of microscopic organisms. Trans. Amer. Micros. Soc. **23**: 103-144. 1902.

Williams, J. Lloyd. ('05). Studies on Dictyotaceæ. The periodicity of the sexual cells in *Dictyota dichotoma.* Ann. Bot. **19**: 531-560. 1905.